The larval and first crab stages of three *Inachus* species (Crustacea: Decapoda: Majidae); a morphological and statistical analysis

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Introduction

Several authors have acknowledged difficulties in distinguishing between congeneric brachyuran crab·larvae (see Lebour, 1928 : 546; Hartnoll, 1961 : 181; Christiansen, 1969; Rice & Ingle, 1975*a* & 1975*b*; Ingle, 1982). These observations were based on a limited amount of material that did not permit statistical analysis of larval characters. Rearing large numbers of crab larvae (see Ingle & Clark, 1977) provided sufficient material for this statistical study. A multivariate technique was used to examine the larvae and first crab stage of three species of spider crabs belonging to the genus *Inachus*.

Material & Methods

Berried females of *Inachus dorsettensis* and *I. phalangium* were collected by trawl from localities off Port Erin, Isle of Man, and Plymouth and *I. leptochirus* was trawled from *Modiolus* beds four miles south of Spanish Head, Isle of Man. The adult females, together with the larvae used in this study are deposited in the BM(NH). Larvae were reared using methods described by Ingle & Clark (1977), then fixed and preserved in 80% alcohol. Twenty specimens of each stage were dissected and mounted as permanent slide preparations in lignin pink/polyvinyl lactophenol. For the multivariate study 43, 142 & 178 characters were used for zoeal, megalop and first crab stages respectively. The majority of these characters are meristic, e.g. setal counts on appendages, but a few were present or absent scores. Accurate measurements of spines and carapace dimensions, as well as carapace setal counts of megalops and crab stages proved to be impracticable and were not used. Setal counts were scored for one side of the body although on occasions it was necessary to combine parts of both left and right appendages to form a complete score. The data was subjected to Principal co-ordinate analysis according to Gower (1966). This method summarizes similarities between OTUs as a 2-dimensional plot. The computation was carried out using a varian V 72 computer. Each larval stage was analysed separately to avoid major differences between stages swamping any specific variation.

Results

Statistical Analysis

First and second principal co-ordinates were plotted for zoea II, megalops and first crab stage of each species. The zoea I stage data was not computed because only two characters were considered significant on inspection. The OTUs in zoea II (Fig. 1) can be separated into two groups, *I. leptochirus* and *I. dorsettensis/I. phalangium* whereas in both megalops (Fig. 2) and first crab stage (Fig. 3) they are clearly separated into three groups which correspond to the three species.

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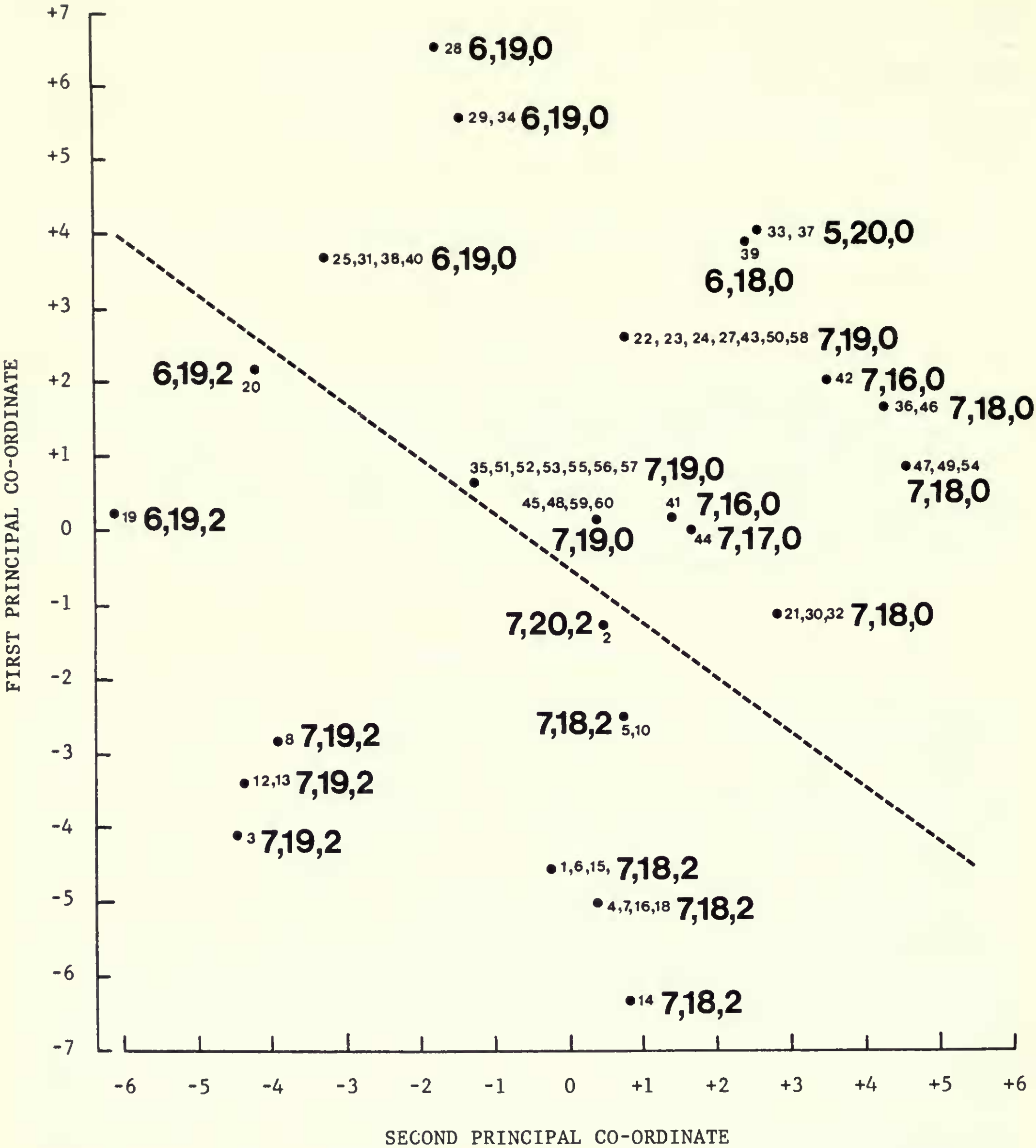

Fig. 1 A plot of the 1st & 2nd Principal co-ordinates of zoea II. Scores of variate 1 (range **5, 6, 7**), variate 11 (range **16, 17, 18, 19, 20**) and variate 36 (range **0, 2**) are plotted against their OTUs. OTUs 1–20 *I. leptochirus*, 21–40 *I. dorsettensis*, 41–60 *I. phalangium*. The dotted line divides the OTUs into two groups, group 1 = *I. leptochirus* and group 2 = *I. dorsettensis/I. phalangium*. Variate 36 is the only diagnostic character separating the two groups. Note that the dotted line has no statistical significance.

The zoea I stages can be divided into two groups, *I. leptochirus* and *I. dorsettensis/I. phalangium*, using the basal article of the second maxilliped and the posterio-dorsal margin of the first abdominal somite. *I. leptochirus* has one seta on the basis (Fig. 4b) and two on the first abdominal somite (Fig. 4c) whereas *I. dorsettensis* and *I. phalangium* have no setae at either site (Fig. 4a, d). The first zoeal stages of *I. dorsettensis* and *I. phalangium* cannot be separated on setal characters.

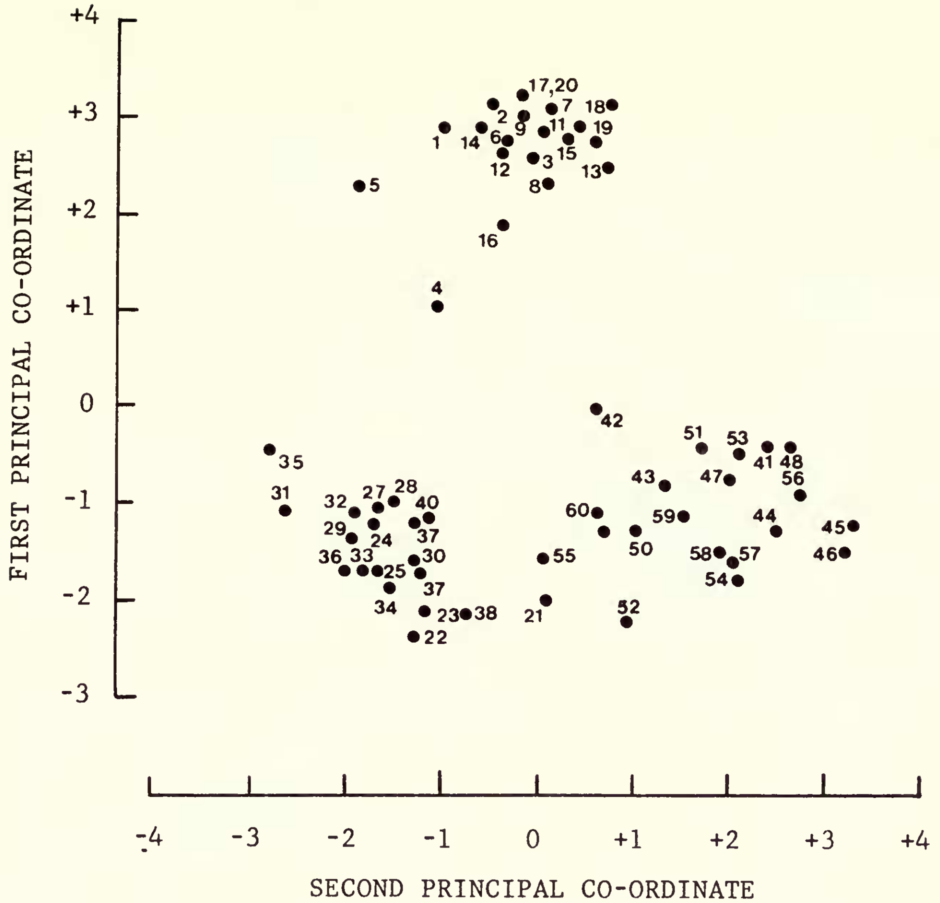

Fig. 2 A plot of the 1st & 2nd Principal co-ordinates of megalops using 68 variates. OTUs 1–20 *I. leptochirus*, 21–40 *I. dorsettensis*, 41–60 *I. phalangium*. These OTUs fall into 3 distinct groups which correspond to the three British *Inachus* species.

The second zoeal stages can also be split into the same two groups (Fig. 1). The three characters showing variation were the number of terminal aesthetascs on the antennule, the numbers of setae on the margin of the maxillary endopod and on the posterio-dorsal margin of the first abdominal somite. The scores of these three characters are shown respectively on Fig. 1 in bold type, adjacent to their respective OTUs. Separation of the two groups shown by the dotted line is determined only by one character, the number of posterio-dorsal marginal setae on the first abdominal somite. *I. leptochirus* has 2 setae (Fig. 4c) whereas *I. dorsettensis* and *I. phalangium* have none (Fig. 4d).

The megalops can be divided into three groups which correspond to the three species using only 68 out of the original 142 characters (Fig. 2). Group separation remains constant however, if only nine characters are used.

By using combinations of the means of these nine characters it seems possible to separate the megalops of the three species.

Nevertheless, their overlapping distribution (Table 2) makes separation very difficult in practice as no single character separates all three species. For example, OTU 21 *(I. dorsettensis)* is grouped with OTUs 52 and 53 *(I. phalangium)* on its overall similarity as they have identical scores for all nine characters. Overlapping ranges of variation in numbers of setae makes it impossible to differentiate all three species with absolute confidence at the

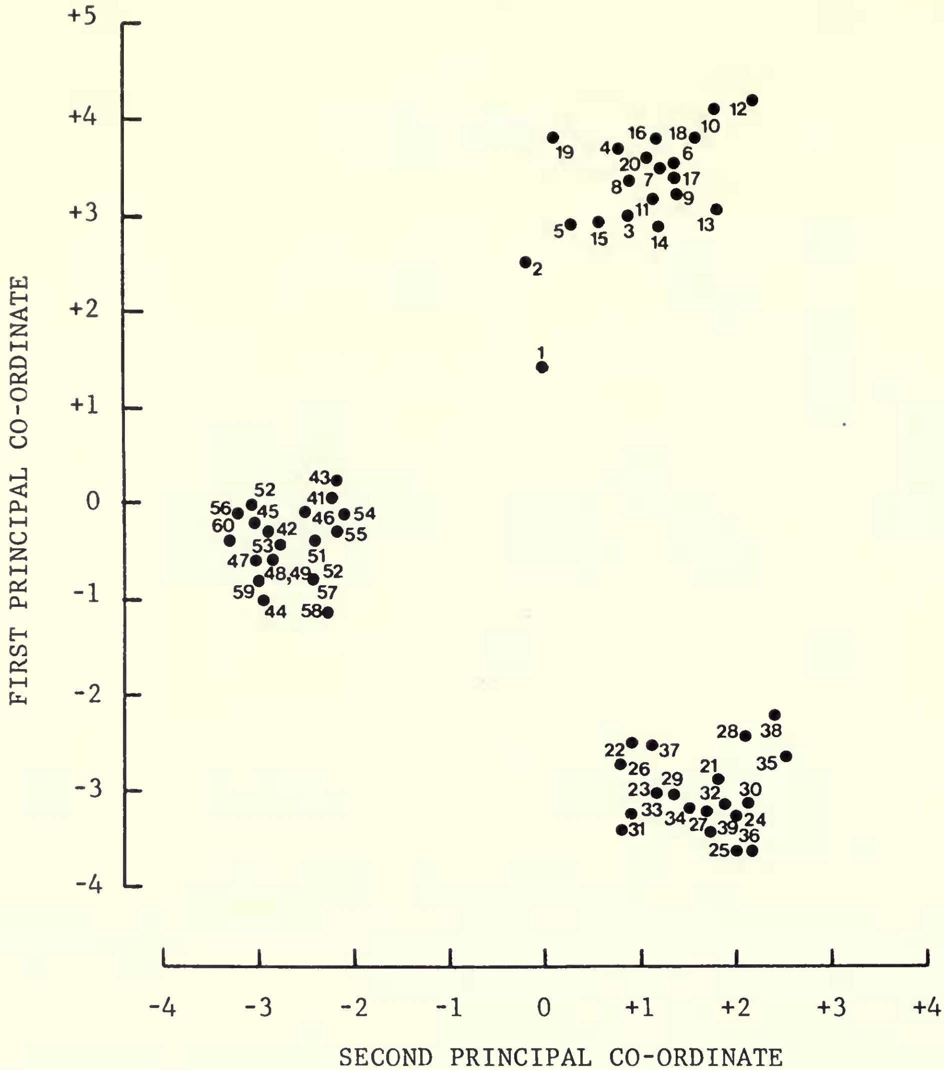

Fig. 3 A plot of the 1st & 2nd Principal co-ordinates of the first crab stages using 138 variates. OTUs 1–20 *I. leptochirus*, 21–40 *I. dorsettensis*, 41–60 *I. phalangium*. These OTUs fall into 3 distinct groups which correspond to the three British *Inachus* species.

megalop stage. Only the number of setae on the proximal exopod segment of the antennule (Figs. 4e, f) displays no intraspecific variability and serves as a diagnostic character separating *I. leptochirus* from *I. dorsettensis/I. phalangium*. The number of spines on the merus of the first peraeopd is also a relatively good character separating *I. leptochirus* from the other two species, but this is a particularly difficult character to observe because of the thickness of the merus.

Differentiation of the first crab stage OTUs (Fig. 3) was achieved using 138 characters from the original 178, but clustering of the OTUs corresponding to the three *Inachus* species does not alter when the number of characters is reduced to 21 (Fig. 5). These 21 characters are listed in Table 3.

As with the megalops, separation of the three species is marked when the means of the variates (Table 4) are used, but again the distributions show considerable overlap. In

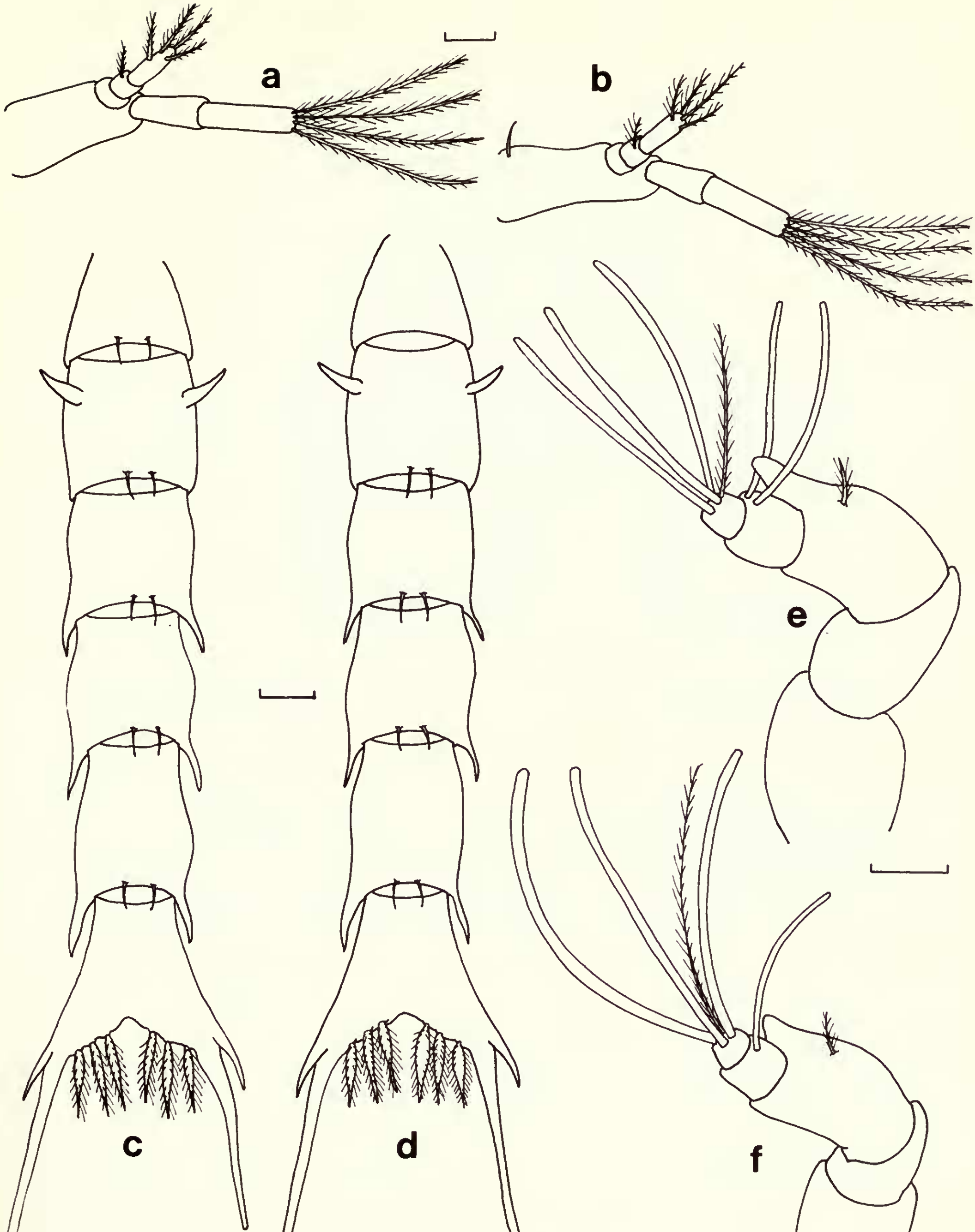

Fig. 4 2nd maxilliped, zoea I (a) *I. dorsettensis* & *I. phalangium* (b) *I. leptochirus*; abdomen of zoea I & II (c) *I. leptochirus* (d) *I. dorsettensis* & *I. phalangium*; antennule of megalop (e) *I. leptochirus* (f) *I. dorsettensis* & *I. phalangium*.

Table 1 A list of characters that may separate the megalops of the three *Inachus* species

Variate no.	Characters
4	number of setae on proximal exopod segment of antennule
7	number of setae on first segment of antenna
44	number of setae on merus of 3rd maxilliped
48	number of setae on epipodite of 3rd maxilliped
62	number of spines on merus of 1st peraeopod
67	number of setae on propodus of 2nd peraeopod
81	number of setae on propodus of 3rd peraeopod
95	number of setae on propodus of 4th peraeopod
109	number of setae on propodus of 5th peraeopod

Table 2 Studying the means of each character from Table 1, the megalops in theory are separable using combinations of characters. However, if the distribution of each character is tabulated only variate 4 is a good diagnostic character, but this only separates *I. leptochirus* from *I. dorsettensis/I. phalangium* (see Figs. 4e, f). (L = *I. leptochirus*, D = *I. dorsettensis* & P = *I. phalangium*)

distribution of variate 4

	1	2	mean
L	0	20	2
D	20	0	1
P	20	0	1

variate 4 separates L from D & P

distribution of variate 7

	0	1	mean
L	0	20	1
D	2	18	1
P	15	5	0

variate 7 separates P from D & L

distribution of variate 44

	4	5	mean
L	1	19	5
D	18	2	4
P	16	4	4

variate 44 separates L from D & P

distribution of variate 48

	0	1	2		mean
L	0	0	7	13	3
D	1	9	8	2	2
P	0	5	10	5	2

variate 48 separates L from D & P

distribution of variate 62

	2	3	4	mean
L	0	0	20	4
D	0	20	0	3
P	4	15	1	3

variate 62 separates L from D & P

distribution of variate 67

	16	17	18	mean
L	3	17	0	17
D	19	1	0	16
P	0	19	1	17

variate 67 separates D from L & P

distribution of variate 81

	15	16	17	mean
L	0	2	18	17
D	1	19	0	16
P	0	3	17	17

Variate 81 separates D from L & P

distribution of variate 95

	15	16	17	mean
L	0	6	14	17
D	4	16	0	16
P	1	10	9	16

variate 95 separates L from D & P

distribution of variate 109

	14	15	16	mean
L	1	2	17	16
D	0	20	0	15
P	1	18	1	15

variate 109 separates L from D & P

Note that the means have been rounded up to the nearest whole number.

Table 3 List of characters that may be used to separate the 1st crab stages

Variate no.	Characters
6	aesthetascs on 2nd exopod segment of antennule
9	setae on endopod of antennule
13	number of setae on 3rd segment of antenna
14	number of spines on 1st segment of antenna
21	number of setae on distal segment of mandibular palp
45	number of setae on basis of 2nd maxilliped
57	number of setae on coxa of 3rd maxilliped
94	number of setae on basis of 2nd peraeopod
99	number of spines on merus of 2nd peraeopod
100	number of spines on ischium of 2nd peraeopod
114	number of setae on ischium of 3rd peraeopod
115	number of setae on basis of 3rd peraeopod
120	number of spines on merus of 3rd peraeopod
121	number of spines on ischium of 3rd peraeopod
135	number of setae on ischium of 4th peraeopod
136	number of setae on basis of 4th peraeopod
141	number of spines on merus of 4th peraeopod
146	number of hooks on propodus of 4th peraeopod
156	number of setae on ischium of 5th peraeopod
157	number of setae on basis of 5th peraeopod
167	number of hooks on propodus of 5th peraeopod

practice, only a combination of characters can be used to distinguish between the species at first crab stage.

Morphology

The general morphology of *I. dorsettensis* was described and illustrated by Ingle (1977). Most of the setal counts fall within the variation recorded during the present study. This indicates little or no temporal variation. Some of the discrepancies may be due to the difficulty of classifying and objectively defining elements, as between a seta and a spine, when the structures grade one into the other (Gurney, 1931 : 38). However, some disparity between Ingle's study and the present work could not be accounted for.

Discussion

Williamson (1965 : 390) listed the presence of a seta on the outer margin of the maxillule basal endite as one of ten characters for separating brachyuran larvae from anomuran larvae. In previous descriptions of zoea II in majids this character is shown as present, for example, Ingle (1977) records this seta as present in zoae II and megalops of *Inachus* reared from Plymouth material — this was confirmed by re-examining Ingle's material and by rearing fresh material from the Plymouth area. In this study the seta was absent from zoea II of *I. dorsettensis* reared from the Isle of Man and from all three megalops.

Lebour* (1928) suggested that larvae of the three *Inachus* species could be separated on size, chromatophore patterns and length of dorsal spines, but none of these claims could be verified. Only the megalops and first crab stage of *I. leptochirus* in the present study proved to be larger than those of the other two species. Larval inachinids have apomorphic zoeal characters which were listed by Rice (1980 : 307), to which can now be added the absence of

*Lebour's material is no longer extant; Ingle, pers. comm.

Table 4 Studying the means of each character from Table 3, the first crab stages may be using a combination of characters. However, if the distribution of each character is tabulated it is evident that no one character is completely diagnostic. (L=*I. leptochirus*, D= *I. dorsettensis* & P=*I. phalangium*)

distribution of variate 6

	1	2	3	mean
L	0	2	18	3
D	18	2	0	1
P	20	0	0	1

variate 6 separates L from P & D

distribution of variate 9

	3	4	mean
L	1	19	4
D	17	3	3
P	18	2	3

variate 9 separates L from P & D

distribution of variate 13

	2	3	4	mean
L	0	19	1	3
D	20	0	0	2
P	0	14	6	3

variate 13 separates D from P & L

distribution of variate 18

	2	3	4	mean
L	0	2	18	4
D	2	9	9	3
P	1	13	6	3

variate 18 separates L from P & D

distribution of variate 21

	0	1	2	mean
L	0	0	20	2
D	0	14	6	1
P	1	19	0	1

variate 21 separates L from P & D

distribution of variate 45

	0	1	mean
L	0	20	1
D	12	8	0
P	17	3	0

variate 45 separates L from P & D

distribution of variate 57

	4	5	6	7	8	9	10	11	mean
L	1	0	0	3	6	4	5	1	8
D	0	5	15	0	0	0	0	0	6
P	0	3	17	0	0	0	0	0	6

variate 57 separates L from P & D

distribution of variate 94

	0	1	2	mean
L	0	2	18	2
D	1	19	0	1
P	0	1	19	2

variate 94 separates D from P & L

distribution of variate 99

	0	1	mean
L	3	17	1
D	19	1	0
P	20	0	0

variate 99 separates L from P & D

distribution of variate 100

	0	1	2	mean
L	0	0	20	2
D	15	4	1	0
P	3	8	8	1

variate 100 separates L from P & D

distribution of variate 114

	3	4	5	6	mean
L	0	1	18	1	5
D	1	18	1	0	4
P	0	0	19	1	5

variate 114 separates D from L & P

distribution of variate 115

	1	2	mean
L	1	19	2
D	20	0	1
P	0	20	2

variate 115 separates D from L & P

distribution of variate 120

	0	1	mean
L	2	18	1
D	19	1	0
P	20	0	0

variate 120 separates L from P & D

distribution of variate 121

	0	1	2	mean
L	1	3	16	2
D	6	12	2	1
P	2	17	1	1

variate 121 separates L from P & D

distribution of variate 135

	4	5	6	mean
L	1	16	3	5
D	20	0	0	4
P	1	17	2	5

variate 135 separates D from P & L

distribution of variate 136

	1	2	3	mean
L	1	19	0	2
D	20	0	0	1
P	3	16	1	2

variate 136 separates D from P & L

distribution of variate 141

	0	1	mean
L	3	17	1
D	18	2	0
P	20	0	0

variate 141 separates L from D & P

distribution of variate 146

	2	3	4	5	mean
L	1	16	3	0	3
D	20	0	0	0	2
P	0	18	1	1	3

variate 146 separates D from L & P

distribution of variate 156

	1	5	6	mean
L	0	17	3	5
D	19	1	0	4
P	1	17	2	5

variate 156 separates D from L & P

distribution of variate 157

	0	1	2	mean
L	0	0	20	2
D	1	19	0	1
P	0	0	20	2

variate 157 separates D from P & L

distribution of variate 167

	2	3	mean
L	0	20	3
D	20	0	2
P	3	17	2

variate 167 separates D from P & L

Note that the means have been rounded up to the nearest whole number.

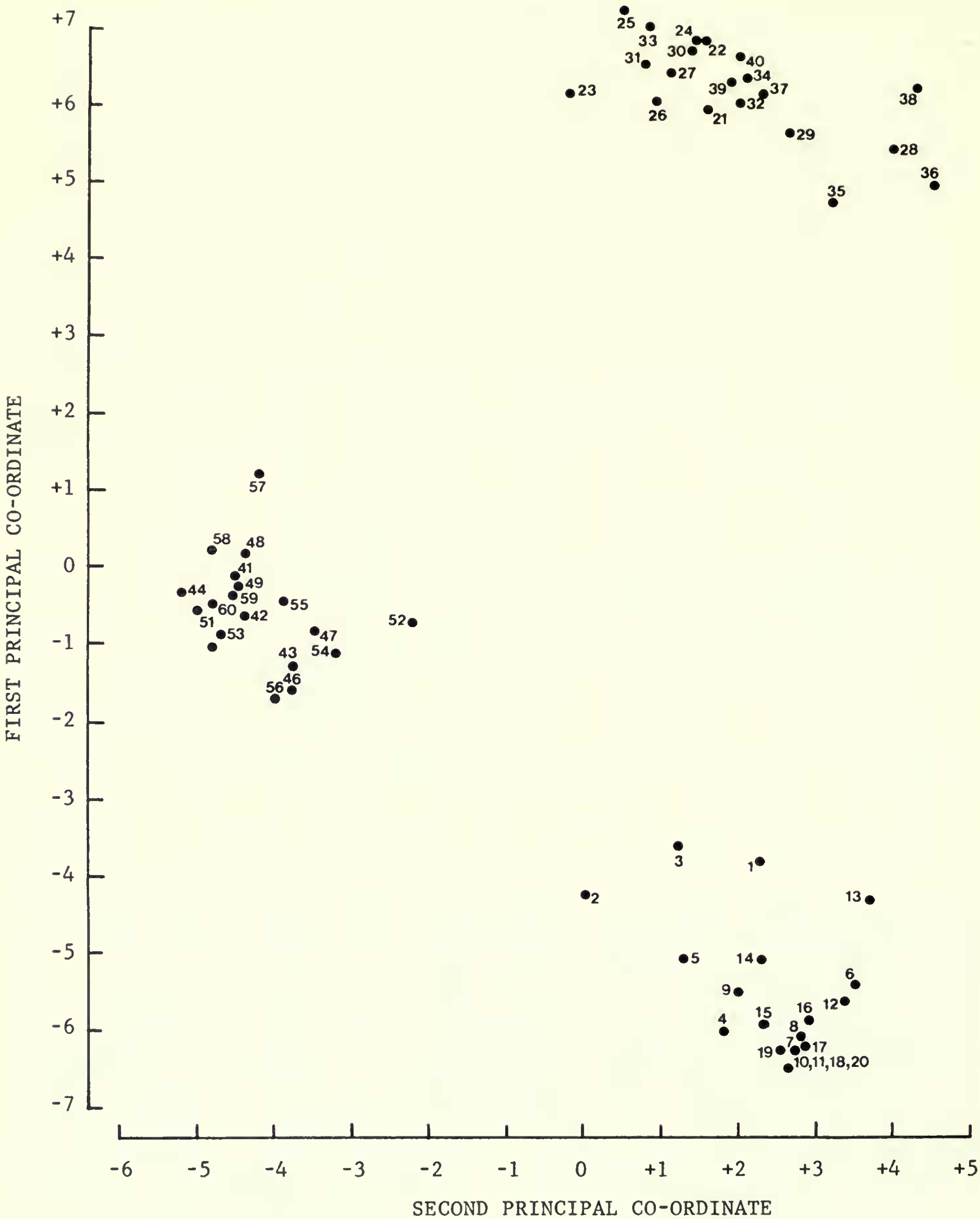

Fig. 5 A plot of the 1st and 2nd Principal co-ordinates of first crab stage using 21 variates. OTUs 1–20 *I. leptochirus*, 21–40 *I. dorsettensis*, 41–60 *I. phalangium*. Although the number of variates is reduced from 138 to 21, the OTUs still fall into 3 distinct groups which correspond to the 3 British *Inachus* species. The grouping of the OTUs is similar to that in Fig. 3 and therefore illustrates that the 21 characters selected contribute to the separation of the 3 groups. Note that the groups *I. leptochirus* and *I. dorsettensis* have changed positions when compared with Fig 3; this has no significance in the analysis.

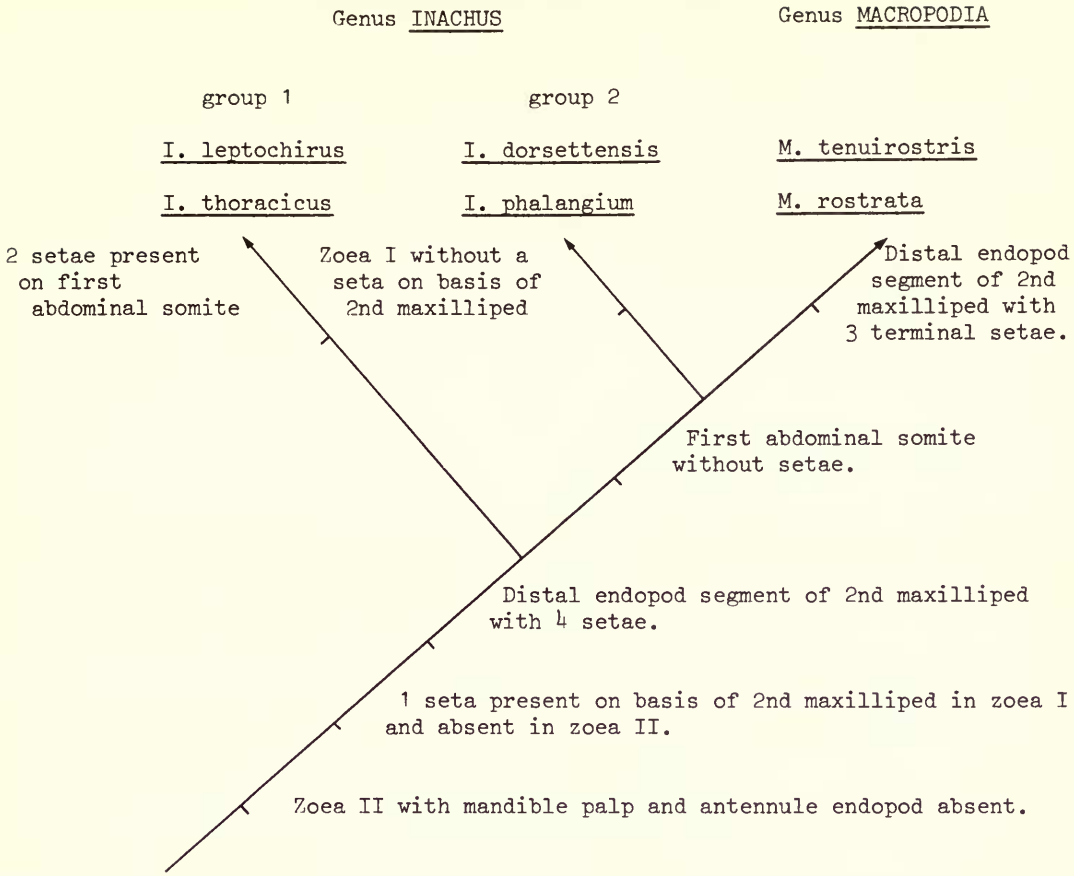

Fig. 6 Cladogram of known *Inachus* & *Macropodia* larval descriptions.

a mandibular palp and endopod bud on the antennule of zoea II. Similarly, when compared with other majid larva characters the loss of paired dorsal setae on the first abdominal somite and the absence of setae on the basis of the 2nd maxilliped in *I. dorsettensis* and *I. phalangium*, can be considered as derived traits. The present study failed to reveal characters which separate the larvae of *I. dorsettensis* from *I. phalangium*, but demonstrated that the larvae of *I. leptochirus* can be easily recognized.

Adult males of *I. leptochirus* share one important feature with two other species of *Inachus* (i.e. *I. thoracicus* & *I. aquiarii)* in having a sternal callosity, a character that is absent in males of *I. dorsettensis, I. phalangium* and *I. communissimus.* Such a separation of *Inachus* species into two groups, those with and those without a sternal callosity, is supported by the present study. Heegaard (1963) studied the zoeae of *I. thoracicus* and clearly figures two setae on the first abdominal somite (p. 475, Fig. 83), but not a seta on the basis of the 2nd maxilliped (p. 475, Fig. 82). Unfortunately Heegaard's material is no longer extant. Re-examination of *I. thoracicus* zoeae may well show that they are inseparable from *I. leptochirus,* adding support to the suggestion that there are two natural groups in the genus *Inachus.*

Present larval evidence supports the view that *Inachus* and *Macropodia* are the most derived of all majids since they show the greatest reduction in numbers of setae; considered by Rice (1980) to be the derived condition. A suggested phylogeny of well documented larvae from the genera *Inachus* and *Macropodia* is shown in Fig. 6.

Setal studies of other brachyuran genera have shown that the larvae of species accepted as closely related are not usually separable on quantitative characters. Therefore meristic setal incongruities within genera, as shown here for *Inachus,* may be the only morphological evidence of phylogenetic non-homogeniety.

This project formed part of an M.Sc. degree in Modern Taxonomy. All relevant data and larval figures were deposited in the Crustacea Section, BM(NH) and the Library of the Polytechnic of Central London.

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References

Christiansen, M. E. 1969. Marine invertebrates of Scandinavia. No. 2. Crustacea, Decapoda, Brachyura. *Universitetsforlaget, Oslo* pp. 1–143. Oslo.

Clark, P. F. 1980. British Spider Crabs of the genus *Inachus;* a morphological study of larval development. M.Sc. Modern Taxonomy (C.N.A.A.) Thesis. Polytechnic of Central London/City of London Polytechnic.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53** (3–4) : 325–358.

Gurney, R. 1931. *British Fresh-Water Copepoda.* Vol. I, 239 pp, Ray Society, London.

Hartnoll, R. G. 1961. A re-examination of the Spider Crab, *Eurynome* Leach from British waters. *Crustaceana* **2** (3) : 171–182.

Heegaard, P. 1963. Decapod larvae from the Gulf of Napoli, hatched in captivity. *Vidensk. Meddr dansk. naturh. Foren.* **125** : 449–493.

Ingle, R. W. 1977. The larval and post-larval development of the Scorpion Spider Crab, *Inachus dorsettensis* (Pennant) (Family: Majidae) reared in the laboratory. *Bull. Br. Mus. nat. Hist.* (Zool.) **30** (9) : 329–348.

—— 1982. Larval and post larval development of the slender-legged Spider Crab, *Macropodia rostrata* (Linnaeus) (Oxyrhyncha: Majidae: Inachinae), reared in the laboratory. *Bull. Br. Mus. nat. Hist.* (Zool.) **42** (3) : 207–225.

Ingle, R. W. & Clark, P. F. 1977. A laboratory Module for rearing crab larvae. *Crustaceana* **32** (2) : 220–222.

Lebour, M. V. 1928. The larval stages of the Plymouth Brachyura. *Proc. zool. Soc. Lond.* **2** : 473–560.

Rice, A. L. 1980. Crab zoeal morphology and its bearing on the classification of the Brachyura. *Trans. zool. Soc. Lond.* **35** : 271–424.

Rice, A. L. & Ingle, R. W. 1975*a.* The larval development of *Carcinus maenas* (L.) and *C. mediterraneus* Czerniavsky (Crustacea, Brachyura, Portunidae) reared in the laboratory. *Bull. Br. Mus. nat. Hist.* (Zool.) **28** (3) : 101–119.

—— —— 1975*b*. A comparative study of larval morphology of the British Portunid crabs *Macropipus puber* (L.) and *M. holstatus* (Fabricius), with a discussion of generic and sub-familial larval characters within the Portunidae. *Bull. Br. Mus. nat. Hist.* (Zool.) **28** (4) : 121–151.

Williamson, D. I. 1965. Some larval stages of three Australian crabs belonging to the families Homolidae and Raninidae, and observations on the affinities of these families (Crustacea: Decapoda). *Aust. J. mar. Freshw. Res.* **16** : 369–398.

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